## REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow. Support for the antibody being human or humanized chimeric is found page 1, line 5 and anti-HLA-DR at page 10, line 32. Also, to follow conventional US claim practice, the word "leukemias" has been amended to "leukemia."

## Drawings

The present application was filed with drawings. Acceptance of these drawings by checking the appropriate boxes in the Notice of Allowability or the Office Action Summary is respectfully requested in the next communication from the examiner. **This applicants' second request for the Examiner's acceptance of the drawings.**

## Rejections Under 35 USC 112, Second Paragraph

The amended claims no longer recite the phrases "the number of antigenic sites or the antigenic density is low" or "optimized human or humanized monoclonal antibody" and therefore the rejections for indefiniteness have been overcome.

## Rejections Under 35 USC 112, First Paragraph, Enablement

The Examiner rejects claims 13 and 14 as failing to comply with the enablement requirement, because the specification does not set forth the antigen specificity of the antibody used to treat chronic myeloid leukaemia (CML). Applicants traverse this rejection for the following reasons.

Attached hereto as Exhibit 1 is Amirzargar et al., Pathology Oncology Research, Vol. 13, No. 1, (2007) pp. 47-51, which evidences that HLA-DR molecules play an important role in the response of the immune system to tumor cells, and that the most frequent haplotypes in CML patients are HLA-DRB1.

Furthermore, the examples of the present specifcation show that an anti-HLA-DR antibody produced by YB2/0 and having the glycannic structure described in the

specification, can induce ADCC against the target cells, which would be useful in the treatment of CML.

The fact that no anti-HLA-DR antibodies has been used, before the filing date of the invention, to treat CML, although the presence of HLA-DR antigens on CML cells was known, can be explained by the insufficiency of biological activity against CML cells of the anti-HLA-DR produced by CHO.

The specificity of the antibody is now recited in the claims, and the working examples of the specification show the effects of the antibodies of the invention comparing to CHO-produced-antibodies. Applicant therefore think that the specification has provided sufficient guidance to practice the present invention without an undue burden.

**Rejections Under 35 USC 112, First Paragraph, Written Description**

The claims no longer recite the words "antigenic" or "derivatives" thereby overcoming the rejection for lack of written description.

**Rejections Under 35 USC 102(b)**

EP 1229125 does not disclose the treatment of CML, therefore this reference cannot inherently anticipate the present invention.

The present claim is a method of treating chronic myeloid leukemia, which is recited in the preamble of the claim. In method of treatment claims, it is proper to treat recitations in preambles as claim limitations. See *Rapoport v. Dement*, 254 F.3d 1053 (Fed. Cir. 2001). The following passage from *Rapoport* at page 1059, analyzing a claim that began "A method for treatment of sleep apneas comprising . . .", is illustrative.

> First, we note that the disputed phrase "treatment of sleep apneas" is technically part of the preamble of the interference count, because it appears before the transition word "comprising." However, there is no dispute in this case that the phrase should be treated as a claim limitation.

The preamble of the present claims does contain a claim limitation that is distinguishable over the prior art.

Also, as noted by the Federal Circuit in the *Rapoport* decision it is legally erroneous to base an argument of inherency on probabilities or possibilities. Thus, even where the same compound was administered by the same route in both the prior art and the contested claims, as in *Rapoport*, the PTO's reviewing court found only mere "possibilities," insufficient to establish a case of inherency. "Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *Rapoport* at 1063, quoting from *Cont'l Can Co. USA, Inc. v. Monsanto Co.*, 948 F.2d 1264 (Fed. Cir. 1991). Therefore, EP '125 cannot inherently render the present anticipated.

## Conclusion

Applicants believe the present claims are allowable.

**Extension Fee and Petition Authorization**

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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EXHIBIT 1

PATHOLOGY ONCOLOGY RESEARCH Vol 13, No 1, 2007

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ARTICLE

# Association of HLA Class II Allele and Haplotype Frequencies with Chronic Myelogenous Leukemia and Age-at-Onset of the Disease

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**Chronic myelogenous leukemia (CML) is characterized by the presence of Philadelphia chromosome resulting from bcr/abl translocation. To clarify the association between HLA class II allele and haplotype frequencies in CML, 50 patients referred to Hematology Oncology and Bone Marrow Transplantation (BMT) center, Shariaty Hospital, Tehran, Iran, were randomly selected and compared with a group of 80 unrelated healthy blood donor subjects. HLA class II alleles were determined by PCR-SSP method. The results showed that the frequencies of DQB1*03011 (P=0.01) and DQA1*0505 (P=0.05) were higher, while that of DQB1*03032 (P=0.04) was lower in patients than in the controls. Regarding age-at-onset, the frequency of HLA-DRB1*07 (P=0.03) and -DQA1*0201 (P=0.03) alleles were higher in patients younger than 35 years. The most frequent haplotypes in our CML patients were HLA-DRB1*11/-DQB1*03011/-DQA1*0505 (P=0.01) and HLA-DRB1*04/-DQB1*0302/-DQA1*03011 (P=0.02). In conclusion, it is suggested that positive and negative association in certain HLA alleles and haplotypes exist in Iranian patients with CML.** (Pathology Oncology Research Vol 13, No 1, 47–51)

*Key words:* chronic myelogenous leukemia, genetic susceptibility, HLA-DRB, HLA-DQA1, HLA-DQB1

## *Introduction*

Chronic myelogenous leukemia (CML) is a malignant hematological disorder that occurs mostly in the fourth and fifth decades of life. In 90% of the patients a translocation exists and can be readily diagnosed by karyotyping. This alteration t(9;22)(q34;q11) results in Philadelphia chromosome formation. Two genes fused together (bcr and abl) encode the P210 fusion protein with a tyrosine kinase activity.[7,15] P210 fusion protein as a novel protein to the immune system should be presented to T cells in association with HLA molecules. In this case HLA molecules play an important role in the response of the immune system to tumor cells.[13,16] In a case-control study designed by Mundhada et al to assess the association of HLA alleles with CML in 163 patients and 376 control subjects, a significant positive association was observed between CML and some alleles.[9] In two other studies HLA-A3, -B8, -DR4 [13] and -Aw19 [2] have been reported as protective markers against CML. In Chinese patients, HLA-DPB1*1301 and -DPB1*20011 frequencies were higher in patients compared to controls.[14] HLA-DRw6 frequency was significantly lower in Sicilian patients than in controls.[4] Dorak et al studied the frequency of HLA alleles and haplotypes in 169 patients of different ages, and their results indicate a role of HLA-DRB3 and -DRB4 homozygosity as a protective and as a risk factor, respectively.[5] Moreover, HLA-DRB4 homozygosity frequency was highest in the early age patients, while these patients had the lowest frequency of HLA-DRB3.[13] In the present study we have analyzed the frequency of HLA-DRB, -DQA1 and -DQB1 alleles and haplotypes in Iranian patients with CML and in a healthy control group. In addition, we have compared the frequency of these alleles in patients of different ages.

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## *Materials and methods*

### *Sampling and DNA extraction*

A total of 50 patients with CML (age 18-69 years, mean 44.6 years, 24 males and 26 females), referred to Hematology Oncology and BMT center at Shariaty Hospital (Iranian referral center for Hematological and Leukemic disorders in Tehran), have been selected for this study. As a control group, 80 unrelated healthy subjects (mean age 32±10 years, 40 males and 40 females) were randomly selected from healthy blood donors admitted to Iranian Blood Transfusion Organization (IBTO). Patients were diagnosed on the basis of cytogenetic studies by the G-banding method and all of them were positive for Philadelphia (Ph) chromosome. Informed consent had been taken from all patients and control subjects participating in this study.

Genomic DNA was extracted from 10 ml peripheral blood in EDTA vacutainers by modified salting-out method.[8] HLA-DRB1, -DQA1 and -DQB1 typing was performed by polymerase chain reaction based on sequence-specific primers (PCR-SSP), following the method of Olerup and Zetterquist.[11] HLA-DRB1, -DQA1 and -DQB1 PCR-SSP kits were supplied by Biotest (Heidelberg, Germany). Taq DNA polymerase was purchased from Roche (Basel, Switzerland). The PCR reactions were carried out in 10 µl volumes. Samples were amplified in Techne genius thermal cyclers, after initial denaturation at 94°C for 2 minutes, followed by 10 cycles of 94°C denaturation for 10 seconds, 65°C annealing and extension for 60 seconds, and finally 20 cycles of 94°C denaturation for 10 seconds, 61°C annealing for 50 seconds and 72°C extension for 30 seconds. After amplification, PCR products were run on an agarose gel, and then the gel was interpreted for specific bands using a UV trans-illuminator. The haplotypes were calculated according to Iranian population specific linkage disequilibrium pattern among HLA-DRB, -DQA, and -DQB alleles.[1]

### *Statistical analysis*

The differences in allele and haplotype frequencies of HLA alleles in the studied groups were analyzed using the Chi-square test for two by two tables after Yates' correction.[6] Fisher's exact 2-tailed correction test was used when necessary. Each allele frequency in CML patients was compared to the same allele in controls. The odds ratio (OR) and its 95% confidence intervals (CI) were calculated using the Instat version 3.06 (GraphPad Software, 2003, San Diego, CA, USA), and a P value of 0.05 or less was considered to be significant.

## *Results*

This study included 50 patients with CML and 80 healthy blood donor volunteers as a control group. All of the HLA loci in patients and controls were consistent with Hardy-Weinberg equilibrium. The frequency of each allele and haplotype are shown in *Tables 1-4*. HLA-DQA1*0505 (P=0.05) and HLA-DQB1*03011 (P=0.01) allele frequencies were significantly increased in our patients compared to controls. Haplotypes more frequent in patients than in controls were HLA-DRB1*11/-DQB1*03011/-DQA1*0505 (33% vs. 19.37%, P=0.01), HLA-DRB1*04/-DQB1*0302/-DQA1*03011 (12% vs. 4.37%, P=0.02) and HLA-DRB1*0101/-DQB1*0501/-DQA1*0104 (3% vs. 0%, P=0.05). On the other hand, three alleles were associated with a reduced risk of developing CML. The frequency of HLA-DRB1*1302 (0% vs. 3.75%, P=0.08), HLA-DQB1*0604 (0% vs. 3.75%, P=0.08) and HLA-DQB1*03032 (0% vs. 4.37%, P=0.04) alleles were decreased in the CML patients.

Homozygosity frequencies were examined for each HLA locus,

*Table 1.* **HLA-DRB1 allele frequencies in Iranian CML patients and controls**

| *DRB1* | *CML (n=50) (100 alleles)* | | *Control (n=80) (160 alleles)* | | *Odds ratio (95% CI)* | *P value* |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | | |
| 0101 | 7 | 7 | 13 | 8.12 | 0.85 (0.33-2.21) | 0.81 |
| 15 | 11 | 11 | 23 | 14.37 | 0.74 (0.34-1.58) | 0.55 |
| 16 | 0 | 0 | 3 | 1.87 | 0.22 (0.01-4.38) | 0.29 |
| 0301 | 9 | 9 | 12 | 7.5 | 1.22 (0.49-3.009) | 0.65 |
| 0302 | 1 | 1 | 0 | 0 | 4.84 (0.19-120.05) | 0.38 |
| 04 | 12 | 12 | 12 | 7.5 | 1.68 (0.72-3.90) | 0.27 |
| 07 | 10 | 10 | 23 | 14.37 | 0.66 (0.30-1.45) | 0.34 |
| 08 | 0 | 0 | 5 | 3.12 | 0.14 (0.007-2.57) | 0.16 |
| 0901 | 0 | 0 | 1 | 0.62 | 0.53 (0.02-13.12) | 1.0 |
| 1001 | 1 | 1 | 4 | 2.5 | 0.39 (0.04-3.58) | 0.65 |
| 11 | 33 | 33 | 39 | 24.37 | 1.53 (0.88-2.65) | 0.15 |
| 12 | 1 | 1 | 0 | 0 | 4.84 (0.19-120.05) | 0.38 |
| 1301 | 6 | 6 | 12 | 7.5 | 0.78 (0.28-2.17) | 0.80 |
| 1302 | 0 | 0 | 6 | 3.75 | 0.11 (0.006-2.12) | 0.08 |
| 1303 | 2 | 2 | 0 | 0 | 8.15 (0.39-171.6) | 0.15 |
| 1401 | 6 | 6 | 7 | 4.37 | 1.40 (0.45-4.28) | 0.57 |
| 1402 | 1 | 1 | 0 | 0 | 4.84 (0.19-120.05) | 0.38 |
| DRB3 | 58 | 58 | 76 | 47.5 | 1.53 (0.92-2.53) | 0.12 |
| DRB4 | 22 | 22 | 36 | 22.5 | 0.97 (0.53-1.77) | 1.0 |
| DRB5 | 12 | 12 | 25 | 15.62 | 0.74 (0.35-1.54) | 0.46 |

showing statistically not significant tendencies for higher prevalence in patients compared to controls in the case of HLA-DR11 (10% vs. 5.43%, P=0.28), HLA-DRB3 (34% vs. 21.1%, P=0.14) and HLA-DRB4 (4% vs. 2.2%, P=0.61).

Regarding age-at-onset in 27 of our patients with available age information, patients were divided into two subgroups, one with patients aged less than 35 and the other with those aged more than 35 years at the disease onset. The remarkable differences between allele frequencies are shown in *Table 5*. The frequencies of HLA-DRB1*07 (21.87% vs. 0%, P=0.03) and HLA-DQA1*0201 (21.87% vs. 0%, P=0.03) alleles were higher in patients younger than 35 years. It is interesting in our study that all HLA-DRB1*07-positive patients with information concerning age of disease onset were younger than 35 years, and out of these patients two subjects were homozygous for HLA-DRB4, suggesting that HLA-DRB1*07 is a strong risk factor for early onset of CML in Iranian patients. Homozygosity for HLA-DRB4 reported previously by Dorak et al was not confirmed in this study; for confirmation, however, a larger sample size would be necessary.

*Table 2.* **HLA-DQA1 allele frequencies in Iranian CML patients and controls**

| *DQA1* | *CML (n=50) (100 alleles)* | | *Control (n=80) (160 alleles)* | | *Odds ratio (95% CI)* | *P value* |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | | |
| 0101 | 4 | 4 | 13 | 8.12 | 0.47 (0.15-1.49) | 0.30 |
| 01021 | 7 | 7 | 20 | 12.5 | 0.53 (0.21-1.29) | 0.21 |
| 0103 | 11 | 11 | 22 | 13.75 | 0.77 (0.36-1.67) | 0.57 |
| 0104 | 10 | 10 | 15 | 9.37 | 1.07 (0.46-2.49) | 1.0 |
| 0201 | 9 | 9 | 21 | 13.12 | 0.65 (0.28-1.49) | 0.43 |
| 03011 | 13 | 13 | 12 | 7.5 | 1.84 (0.80-4.22) | 0.19 |
| 0401 | 0 | 0 | 5 | 3.12 | 0.14 (0.007-2.57) | 0.16 |
| 0505 | 35 | 35 | 37 | 23.12 | 1.79 (1.03-3.11) | 0.05 |
| 05011 | 11 | 11 | 15 | 9.37 | 1.19 (0.53-2.72) | 0.68 |

*Table 3.* **HLA-DQB1 allele frequencies in Iranian CML patients and controls**

| *DQB1* | *CML (n=50) (100 alleles)* | | *Control (n=80) (160 alleles)* | | *Odds ratio (95% CI)* | *P value* |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | | |
| 0201 | 18 | 18 | 30 | 18.75 | 0.95 (0.49-1.81) | 1.0 |
| 0203 | 0 | 0 | 1 | 0.62 | 0.53 (0.02-13.12) | 1.0 |
| 03011 | 38 | 38 | 37 | 23.12 | 2.03 (1.18-3.51) | 0.01 |
| 03012 | 0 | 0 | 3 | 1.87 | 0.22 (0.01-4.38) | 0.29 |
| 0302 | 10 | 10 | 7 | 4.37 | 2.43 (0.89-6.60) | 0.12 |
| 03032 | 0 | 0 | 7 | 4.37 | 0.10 (0.005-1.80) | 0.04 |
| 0305 | 1 | 1 | 2 | 1.25 | 0.80 (0.07-8.92) | 0.67 |
| 0306 | 0 | 0 | 1 | 0.62 | 0.53 (0.02-13.12) | 1.0 |
| 0401 | 1 | 1 | 4 | 2.5 | 0.39 (0.04-3.58) | 0.65 |
| 0501 | 13 | 13 | 22 | 13.75 | 0.94 (0.45-1.96) | 1.0 |
| 05031 | 4 | 4 | 9 | 5.62 | 0.69 (0.21-2.33) | 0.77 |
| 06011 | 5 | 5 | 11 | 6.87 | 0.71 (0.24-2.12) | 0.61 |
| 0602 | 10 | 10 | 20 | 12.5 | 0.78 (0.35-1.74) | 0.69 |
| 0604 | 0 | 0 | 6 | 3.75 | 0.12 (0.006-2.12) | 0.08 |

***Discussion***

The P210 fusion protein encoded by t(9;22)(q34;q11) genetic alteration should be recognized by the immune system as an endogenous protein. Although originally it was thought that endogenous intracellular proteins, like P210, would only be presented by HLA class I molecules and exogenous proteins by HLA class II molecules, more recent evidence illustrates that HLA class II molecules also carry fragments of endogenous proteins. Reports have been published on both negative and positive associations of bcr-abl transcripts with HLA class I and class II alleles. Recently it has been reported that HLA class II alleles such as HLA-DR1, -DR2, -DR3, -DR4 and -DR11 can present endogenous proteins like synthetic bcr-abl peptides and induce the generation of T cell responses.[3,12] Accordingly, we have investigated HLA-DRB1, -DQA1 and -DQB1 allele and haplotype frequencies in Iranian CML patients and controls.

The results of our study showed that, although statistically not significant, HLA-DRB1*11 was more frequent in patients than in the control group (33% vs. 24.37%, P=0.15). In contrast, in a Turkish population, HLA-DR11 was considered as a protective marker.[5] These population differences may be attributable to ethnicity. Posthuma et al claimed that HLA-DR4 diminishes the risk of CML.[13] However, our study did not support such a role of HLA-DR4, since it showed a statistically not significant trend to be more frequent in patients than in the control group (12% vs. 7.5%, P=0.27). In our patients, HLA-DRB1*1302 could not be detected, while it was found in 3.75% of controls (P=0.08). Similar results were observed in Japanese patients.[15]

*Table 4.* **HLA class II haplotype frequencies in Iranian CML patients and controls**

| *HLA-DRB1/ -DQB1/-DQA1* | *CML (n=50) (100 alleles)* | | *Control (n=80) (160 alleles)* | | *Odds ratio (95% CI)* | *P value* |
|---|---|---|---|---|---|---|
| *Haplotypes* | *n* | *%* | *n* | *%* | | |
| 0101/0501/0101 | 4 | 4 | 9 | 5.62 | 0.69 (0.20-2.33) | 0.77 |
| 0101/0501/0104 | 3 | 3 | 0 | 0 | 11.52 (0.58-225.6) | 0.05 |
| 15/0602/01021 | 7 | 7 | 9 | 5.62 | 1.26 (0.45-3.50) | 0.79 |
| 15/06011/0103 | 4 | 4 | 9 | 5.62 | 0.69 (0.20-2.33) | 0.77 |
| 0301/0201/05011 | 9 | 9 | 12 | 7.5 | 1.22 (0.49-3.009) | 0.65 |
| 04/0302/03011 | 12 | 12 | 7 | 4.37 | 2.98 (1.13-7.85) | 0.02 |
| 07/0201/0201 | 10 | 10 | 17 | 10.62 | 0.93 (0.41-2.13) | 1.0 |
| 11/03011/0505 | 33 | 33 | 31 | 19.37 | 2.05 (1.15-3.63) | 0.01 |
| 1301/0602/0103 | 6 | 6 | 8 | 5 | 1.21 (0.41-3.60) | 0.78 |
| 1302/0604/0104 | 0 | 0 | 6 | 3.75 | 0.12 (0.006-2.12) | 0.08 |
| 1401/05031/0104 | 6 | 6 | 7 | 4.37 | 1.39 (0.46-4.28) | 0.57 |

*Table 5.* **HLA class II allele frequencies in CML patients aged more vs. less than 35 years**

| *HLA alleles* | *< 35 years (n=16) (32 alleles)* | | *> 35 years (n=11) (22 alleles)* | | *Odds ratio (95% CI)* | *P value* |
|---|---|---|---|---|---|---|
| | *n* | *%* | *n* | *%* | | |
| -DRB1*07 | 7 | 21.87 | 0 | 0 | 13.23 (0.71-245.16) | 0.03 |
| -DRB1*1401 | 0 | 0 | 2 | 9.09 | 0.12 (0.00-2.76) | 0.16 |
| -DRB4 | 12 | 40 | 3 | 15.78 | 3.8 (0.92-15.6) | 0.07 |
| -DQA1*0101 | 0 | 0 | 2 | 9.09 | 0.12 (0.00-2.76) | 0.16 |
| -DQA1*0104 | 2 | 6.25 | 4 | 18.18 | 0.30 (0.05-1.8) | 0.21 |
| -DQA1*0201 | 7 | 21.87 | 0 | 0 | 13.23 (0.71-245.16) | 0.03 |
| -DQB1*0201 | 8 | 25 | 2 | 9.09 | 3.33 (0.63-17.52) | 0.17 |
| -DQB1*0501 | 3 | 9.37 | 5 | 22.72 | 0.35 (0.07-1.66) | 0.24 |
| -DQB1*05031 | 0 | 0 | 2 | 9.09 | 0.12 (0.00-2.76) | 0.16 |

In our population, HLA-DQA1*0505 (35% vs. 23.12%, P=0.05) and HLA-DQB1*03011 (38% vs. 23%, P=0.01) alleles were shown to have positive association with CML. In another study by Mundhada et al a significant positive association was observed with HLA-DQB1*0402, -DQB1*0609 and -DQB1*0201 for b2a2, with HLA-DQB1*0609 for b3a2 and with HLA-DQB1*0502 for e1a2 transcripts. HLA-DQB1*0604 showed a negative association in our patients (0% vs. 3.75%, P=0.08), whereas Mundhada et al reported negative association of HLA-DQB1*0303 and -DQB1*0603 with b2a2 and of HLA-DQB1*0303 with b3a2 transcripts.[9] As a result it is suggested that HLA-DQB1 alleles have different capabilities to present the P210 fusion protein.

We examined homozygosity at each HLA locus in patients and controls. Homozygosity associations for HLA-DR11 (10% vs. 5.43%, P=0.28), HLA-DRB3 (34% vs. 21.1%, P=0.14) and HLA-DRB4 (4% vs. 2.2%, P=0.61) were not statistically significant.

HLA-DRB1, -DQA1 and -DQB1 allele frequencies were analyzed in two groups of our patients, divided according to age at onset, as higher or lower than 35 years. We obtained a significant positive association of HLA-DRB1*07 and -DQA1*0201 with early age of disease onset. It seems that HLA-DRB1*07 is preferentially expressed in cases with early age-at-onset of disease in Iranian patients, because seven out of ten HLA-DRB1*07-positive patients aged less than 35 years whereas the frequency of this allele among 11 patients older than 35 years was zero. Since we did not have sufficient age data, we could not examine the age-specific distribution of homozygous genotypes for DRB3 and DRB4 to replicate previous findings.[5,10] It is suggested that more studies are needed in different ethnic groups to show the homozygosity at different HLA loci and to investigate the risk of CML in individuals with a certain allele or genotype. Knowledge of HLA association with different bcr-abl transcripts would have diagnostic and prognostic implications. It would also help to improve strategies of immunization with bcr-abl peptides against t(9;22) (q34;q11) leukemia.

In conclusion, HLA allele and haplotype associations in our population show some differences from previously published studies on CML. Differences in ethnic background could be behind such controversies.

***Acknowledgement***

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***References***

1. *Amirzargar A, Mytilineos J, Farjadian S, et al*: Human leukocyte antigen class II allele frequencies and haplotype association in Iranian normal population. Hum Immunol 62:1234-1238, 2001
2. *Bortin MM, D'Amaro J, Bach FH, et al*: HLA associations with leukemia. Blood 70: 227-232, 1987

3. *ten Bosch GJA, Toornvliet AC, Friede T, et al*: Recognition of peptides corresponding to the joining region of p210BCR-ABL protein by human T cells. Leukemia 9: 1344-1348, 1995
4. *Caruso C, Lo Campo P, Botindari C, Modica MA*: HLA antigens in Sicilian patients affected by chronic myelogenous leukaemia. J Immunogenet 14: 295-299, 1987
5. *Dorak MT, Chalmers EA, Gaffney D, et al:* Human major histocompatibility complex contains several leukemia susceptibility genes. Leuk Lymphoma 12: 211-222, 1994
6. *Haldane JB*: The estimation and significance of the logarithm of a ratio of frequency. Ann Hum Genet 20:309-311, 1956
7. *Kurzrock R, Kantarjian HM, Druker BJ, et al*: Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. Ann Intern Med 138: 819-830, 2003
8. *Miller SA, Dykes DD, Polesky HF*: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215, 1988
9. *Mundhada S, Luthra R, Cano P*: Association of HLA Class I and Class II genes with bcr-abl transcripts in leukemia patients with t(9;22) (q34;q11). BMC Cancer 4: 25, 2004
10. *Oguz FS, Kalayoglu S, Diler AS, et al*: HLA system affects the age-at-onset in chronic myeloid leukemia. Am J Hematol 73: 256-262, 2003
11. *Olerup O, Zetterquist H*: HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 39: 225-235, 1992
12. *Pawelec G, Max H, Halder T, et al*: BCR/ABL leukemia oncogene fusion peptides selectively bind to certain HLA-DR alleles and can be recognized by T cells found at low frequency in the repertoire of normal donors. Blood 88: 211-218, 1996
13. *Posthuma EFM, Falkenburg JHF, Apperley JF, et al*: HLA-DR4 is associated with a diminished risk of the development of chronic myeloid leukemia (CML). Leukemia 14: 859-862, 2000
14. *Tian H, Zhou SY, Liu ZH, et al*: Association of HLA-DPB1 alleles with chronic myelogenous leukemia in southern Chinese Hans. Zhongguo Shi Yan Xue Ye Xue Za Zhi 11: 266-268, 2003
15. *Yasukawa M, Ohminami H, Kojima K, et al*: Analysis of HLA-DRB1 alleles in Japanese patients with chronic myelogenous leukemia. Am J Hematol 63: 99-101, 2000
16. *Yotnda P, Firat H, Garcia-Pons F, et al*: Cytotoxic T cell response against the chimeric p210 BCR-ABL protein in patients with chronic myelogenous leukemia. J Clin Invest 101: 2290-2296, 1998